

PCT

WORLD INTELLECTUAL PRO  
InternationalDocument No. FP4  
Appl. No. To be assigned

INTERNATIONAL APPLICATION PUBLISHED UNDER

WO 9606850A1

(51) International Patent Classification 6 : <b>C07H 1/08, G01N 33/543, B01D 15/00</b>		A1	(11) International Publication Number: <b>WO 96/06850</b>
			(43) International Publication Date: 7 March 1996 (07.03.96)
(21) International Application Number: PCT/EP95/03385		(81) Designated States: AU, CA, FI, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 28 August 1995 (28.08.95)			
(30) Priority Data: 9401391 29 August 1994 (29.08.94) NL		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71) Applicant (for all designated States except US): AKZO NOBEL N.V. [NL/NL]; Velperweg 76, NL-6824 BM Arnhem (NL).			
(72) Inventors; and			
(75) Inventors/Applicants (for US only): BORST, Govert, Arnoldus, Petrus [NL/NL]; Van Miertstraat 34, NL-5262 XC Vught (NL). ZIGMANS, Johannes, Antonius, Gerardus, Wilhelmus [NL/NL]; Nozemanstraat 51 A, NL-3023 TM Rotterdam (NL). CHEMELLI, Benjamin, Joseph [US/US]; 921 Prologue Road, Durham, NC 27712 (US). ROBINSON, Donald [US/US]; 1942 Long Boat Drive, Lakeland, FL 33809 (US).			
(74) Agent: BEETZ, T.; Postbus 20, NL-5340 BH Oss (NL).			
(54) Title: DEVICE FOR USE IN THE ISOLATION OF A BIOLOGICAL MATERIAL SUCH AS NUCLEIC ACID			
(57) Abstract			
<p>The present invention relates to the isolation of a biological material, for example nucleic acid, from a basic material containing said biological material. A device is provided characterized by a container (1) for holding a mixture of the basic material, a chaotropic substance and a solid phase which binds the biological material; means (10) for separating the solid phase with the biological material from the fluid bound thereto; and means for connecting the container (1) to an inlet (6) and outlet (9) for washing fluid for washing the biological material bound to the solid phase, to an inlet (6) for an eluent fluid, and to an eluate reservoir (17) for collection of the eluant fluid with the dissolved biological material.</p>			
BEST AVAILABLE COPY			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

DEVICE FOR USE IN THE ISOLATION OF A BIOLOGICAL MATERIAL  
SUCH AS NUCLEIC ACID

5       The present invention relates to the isolation of  
a biological material, for example nucleic acid, from a  
basic material containing said biological material.

10       An example of a method for the isolation of  
nucleic acid is known from US-A-5,234,809. In the case of  
this method the basic material, a chaotropic fluid and  
silica particles are mixed, with the result that the  
nucleic acid is adsorbed on the silica particles. The  
silica particles are then separated from the fluid and  
treated with a buffered eluant, in which the nucleic acid  
is dissolved off the particles. With this method HIV  
15 tests, for example, can be prepared by isolating the  
nucleic acid from the basic material, which is blood.

The object of the present invention then is to  
provide a device which is suitable for carrying out the  
methods described and similar methods.

20       To this end, the device according to the inven-  
tion is characterized by a container for holding a  
mixture of the basic material, a chaotropic fluid and a  
solid phase which binds the biological material; means  
for separating the solid phase with the biological  
25 material from the fluid bound thereto; and means for  
connecting the container to an inlet and outlet for  
washing fluid for washing the biological material bound  
to the solid phase, to an inlet for an eluant fluid, and  
to an eluate reservoir for collection of the eluant with  
30 the dissolved biological material.

If the solid phase which binds the biological  
material consists of particle material, then it is  
advantageous if the means for separating the solid phase  
from the fluid are provided with a filter for allowing  
35 through the fluid and retaining the particle material.

The means for connecting the container to the  
inlet or outlet for eluant fluid and washing fluid and/or  
to the eluate reservoir are preferably provided with a

shut-off element, which can be provided with, for example, a septum and an outlet channel for allowing through fluid from the separating means to the outlet, and also a hollow needle element connecting to the  
5 separating means, for piercing the septum in order to connect the separating means to the eluate reservoir.

Such a shut-off element is simple, user-friendly and reliable in operation.

10 In a preferred embodiment of the device according to the invention the container is provided with two sections lying one above the other and separated by a constriction, and is also provided with a supply element which can be connected to the inlet and is movable between a position above the constriction and a position  
15 connecting in a close fit to the constriction.

In this embodiment both compressed gas for discharging the sample fluid from the container and the washing fluid and the eluant fluid can be fed into the container by one and the same supply element. During the  
20 infeed of gas the supply element will be in the position above the constriction, following which the supply element is moved to the position connecting suitably to the constriction, so that only the bottom part of the container, containing the solid phase with the biological  
25 material bound thereto, need be flushed, with the result that less washing fluid is needed and there is less of a risk of contamination occurring.

In an alternative embodiment the shut-off element is a valve element designed with at least one non-return  
30 valve between inlet and container.

In a more advanced development thereof the valve element is provided with three non-return valves: the first-mentioned non-return valve in the connection to a compressed gas supply, a second non-return valve between  
35 the container and the separating means, and a third non-return valve in a connection between the separating means and the inlet for the washing fluid and the eluant.

Compared with the embodiment with one valve, this embodiment has the advantage that the washing fluid is

introduced directly into the separating means, and the container therefore does not need to be washed, with the result that only a small amount of washing fluid is needed.

5           The invention will be explained below with reference to the drawings, which show a number of exemplary embodiments of the device according to the invention.

10           Fig. 1 is a vertical longitudinal section of a first embodiment of the device according to the invention.

          Fig. 2 is a section corresponding to Fig. 1, in which the device according to the invention is placed in a control apparatus.

15           Fig. 3 shows on a larger scale the detail III from Fig. 2, with the supply element in the bottom position.

          Fig. 4 is a vertical section of an alternative embodiment of a part of the device according to Fig. 1, in which the shut-off element is in the washing position.

20           Fig. 5 is a section corresponding to Fig. 4, with the shut-off element in the elution position.

          Fig. 6 shows on a larger scale the detail VI from Fig. 5.

25           Fig. 7 is a vertical longitudinal section of a second embodiment of the device according to the invention.

30           The drawings show exemplary embodiments of a disposable device for use in the isolation of a biological material, such as nucleic acid, from a basic material containing said biological material. The basic material can be, for example, blood, blood serum, urine, faeces, cell cultures and the like. The isolation of the biological material, in particular nucleic acid, is necessary for carrying out tests, such as, for example, an HIV test.

35

          The device shown in Figs. 1 and 2 comprises a container 1 for holding a mixture of the basic material, a chaotropic substance and a solid phase which binds the nucleic acid, in this exemplary embodiment silica

particles. What is meant by chaotropic substance is any substance which is capable of altering the secondary, tertiary and/or quaternary structure of proteins and nucleic acid, but leaves at least the primary structure intact. Examples thereof are guanidine, (iso)thiocyanate and guanidine hydrochloride. In this exemplary embodiment the container 1 belongs specifically to the device and the sample to be examined must be placed in the container by pipetting. The container is then sealed with a cover 2. The cover 2 is designed with an inlet connection 3, for connection of the container 1 to an inlet (not shown) for compressed air, washing fluid and eluant fluid. These inlets for fluids form part of a control apparatus, parts of which are shown in Fig. 2 and in which the device according to the invention can be placed for carrying out the isolation of the nucleic acid. Fig. 2 shows, for example, a connecting ring 4 for suspending the device in the apparatus, for which purpose the container 1 is designed with a circular flange 5. Fig. 2 also shows a connecting element 6 for the inlets for the fluids, which connecting element can be connected to the inlet connection 3 of the cover 2.

The container 1 forms the top element of the device, which is connected at the bottom end to a bottom element 7. This cylindrical bottom element 7 comprises on the periphery an outlet connection 8 for connecting the device to an outlet for sample fluid and washing fluid, which forms part of the apparatus and is indicated by 9. Clamped between the top end of the bottom element 7 and the container 1 is a membrane 10, which serves as a filter and on which the silica particles with nucleic acid adsorbed thereon can settle. A channel 11 connects to the space below the membrane 10. The channel 11, which forms the passage for a needle 12, comes out in a shut-off element 13, which in this case is provided with a septum 20. The shut-off element 13, of silicone material, is provided with an outlet channel 14 with a top part lying in line with the channel 11 and a bottom part running towards the periphery. At the periphery of the

shut-off element 13 the outlet channel 14 opens out into an annular peripheral channel 15 which can be placed in communication with the outlet connection 8 in the bottom element 7. In the position of the shut-off element 13 shown in Fig. 2 sample fluid and washing fluid can be conveyed out of the container 1, by way of the membrane 10 and the channels 11 and 14 to the outlet 9. A removable sealing plate 16 ensures that the outlet connection 8 is sealed before the device is used. The shut-off element could also be placed initially in a closed position and pushed to a discharge position only when the sample fluid is to be discharged.

The device according to the invention also comprises an eluate reservoir 17 for the collection of an eluant supplied from the inlet connection 3, and containing the nucleic acid dissolved off the silica particles. The eluate reservoir can be a standard cup with a capacity of, for example, 0.5 ml, which is shut off by a septum 18 of silicone material. The eluate reservoir 17 can be placed in a positioning element 19 of the apparatus, and with this positioning element 19 eluate reservoir 17 and shut-off element 13 can be pushed up relative to the bottom element 7 with the needle 12, in such a way that the needle cuts open in a sealed-off manner the septum 20 in line with the top part of the outlet channel 14 in the shut-off element 13 and the septum 18 of the eluate reservoir 17, following which eluate supplied can pass into the eluate reservoir 17 without the risk of leakages. A vent channel 21 together with a vent groove in the periphery of the needle 12 (see Fig. 6) ensure that air can escape from the eluate reservoir 17 for the admission of the eluant fluid. The vent channel 21 in the shut-off element 13 can also be combined with the discharge channel 14, while a second needle can also be disposed in the shut-off element for the venting.

It can also be seen in Figs. 1, 2 and 3 that a hollow pin-shaped inlet element 23 is formed in line with the inlet connection 3 of the cover 2, which inlet element projects until it is deep inside the container

1 and is movable in the container 1 through the special construction of the cover with flexible ridges 24. The container 1 is formed in two parts, namely a top part 25 of large volume tapering downwards towards a constriction 24, and a bottom part 26 of small volume and flaring out slightly downwards from the constriction 24, and connecting to the membrane 10. The inlet element 23 can be moved by means of the connecting element 6 of the apparatus between a top position shown in Figs. 1 and 2, in which the inlet element 23 opens out above the constriction 24 in the top part 25 of the container 1, and a bottom position, in which the feed element engages in a shut-off manner in the constriction 24 and therefore opens out in the bottom part 26 of the container 1. The inlet element 23 and/or the constriction 24 could be provided with snap means 27 for reliably maintaining the grip.

The device according to Figs. 1 - 3 works as follows:

First of all, the mixture of the basic material, the chaotropic substance and the silica particles is placed in the container 1, and the sealed device is then placed in the apparatus in the position shown in Fig. 2. Air is then pumped through the inlet connection 3 and the inlet element 23 into the container 1, in order to build up pressure in the container 1 for promoting the discharge of the sample fluid from it. After this discharge of the sample fluid, only the silica particles with adsorbed material remain behind on the membrane 10, together with residues of the sample fluid. The inlet element 23 is then moved to the bottom position in engagement with the constriction 24, following which a washing buffer (mixture of salts), ethanol and acetone are fed in through the inlet element, in order to wash the silica particles and the cavities and passages of the device in question. Air can also be pumped through intermittently, in order to achieve an additional scraping effect. Finally, conditioned warm air is passed through. The next step is then to move up the positioning element 19, in order to move the eluate reservoir 17 and



- 7 -

the shut-off element 13, so that the septums 18 and 20 can be pierced by the needle 12, as a result of which the container 1 enters into communication with the eluate reservoir 17 by way of the filter channel 11. Finally, the eluant fluid, for example in the form of TE buffer, double distilled water or PCR buffer, is fed in through the inlet element 23. The eluant fluid is kept in contact with the silica particles for a predetermined period, following which the eluant fluid is pumped further and passes by way of the membrane 10 and the channel 11 in a predetermined quantity, for example 100  $\mu$ l, into the eluate reservoir 17. In this eluant fluid the nucleic acid is dissolved off the silica particles and is ready for testing. The shut-off element 13 and the eluate reservoir 17 are then moved down again, with the result that the needle 12 returns to the discharge position. Remaining eluant fluid is then pumped away to the outlet. When the needle 12 is withdrawn the septum 18 closes automatically, so that a sealed reservoir 17 with the fluid to be examined is obtained.

Figs. 4 - 6 show a variant of the shut-off element 13 and the eluate reservoir 17, which in this case are combined to a fixed unit and together can be moved between the washing or discharge position shown in Fig. 4 and the elution position shown in Fig. 5, moved upwards relative to the position shown in Fig. 4, in which elution position the needle 12 has pierced through the septum 20 between the outlet channel 14 and the eluate reservoir 17. Fig. 6 shows the abovementioned vent groove 22 in the needle, which ensures that air can escape from the eluate reservoir 17 when the eluant fluid flows into the reservoir. For the rest, the device can be comparable to that of Figs. 1 - 3.

Fig. 7 shows an exemplary embodiment of the device according to the invention which is different in principle. In this case the shut-off element is a valve element 28 on which a standard sample tube can be fitted as container 1. The valve element 28 in this exemplary embodiment contains three commercially available

non-return valves: a non-return valve 29 in a connecting channel 30 between a compressed gas inlet (not shown) and the container 1, a second non-return valve 31 between the container 1 and filter membrane 10 and channel 11 acting  
5 as separating means, and a third non-return valve 32 in a connecting channel 33 between the filter membrane 10 and the channel 11 and the inlet for the washing fluid and the eluant fluid (not shown).

10 Instead of a pierceable septum in the shut-off element, the valve element 28 is provided below the membrane 10 and the channel 11 with a rotary valve 34 for connecting the channel 11 as desired to the outlet connection 8 and the eluate reservoir 17 connected to the valve element 28.

15 This device works as follows:

After the device has been placed in the apparatus belonging to it and the various inlets and outlets are connected, compressed air is supplied to the connecting channel 30, which compressed air passes by way of the  
20 non-return valve 29 into the container 1, and due to the pressure built up therein, the mixture of basic material, chaotropic substance and silica particles present therein is forced through the non-return valve 31 into the valve element 28, where the mixture is filtered and the silica  
25 particles remain behind on the membrane 10, and the fluid passes through the channel 11 and the rotary valve 34 into the outlet connection 8 and the connected outlet. The non-return valve 32 in this case remains closed, so that no fluid can pass into the connecting channel 33.  
30 Washing fluids are then introduced by way of the connecting channel 33 and the non-return valve 32 directly into the valve element 28 with the filter membrane 10, which can be washed with a relatively small volume of washing fluid. The non-return valve 31 ensures that a seal is  
35 provided relative to the container 1. After the rotary valve 34 has rotated in order to produce the connection between the channel 11 and the eluate reservoir 17, eluate is fed through the connecting channel 33 and the non-return valve 32 to the valve element 28, and the

- 9 -

eluant fluid with nucleic acid dissolved therein passes through the membrane 10 with silica particles and by way of the channel 11 into the eluate reservoir.

5 The invention is not restricted to the exemplary  
embodiments shown in the drawing and described above,  
which can be varied in various ways within the scope of  
the invention. For example, mention is always made above  
of the sample fluid being discharged by means of com-  
pressed air or another compressed gas, but it is, of  
10 course, also possible to force the sample fluid out of  
the container 1 by mechanical means. For example, a  
hollow plunger could be provided in the essentially  
cylindrical container, in which case a small channel in  
the plunger is initially shut off by a seal and through  
15 the downward movement thereof in the container, the fluid  
in the container is pressed out through the filter. In  
the bottom position, the seal can then be pierced,  
following which the washing fluids and finally the eluant  
fluid can be supplied through the channel connected to  
20 the inlet in the plunger. Alternatively, it is also  
possible to fit a second plunger in the channel in the  
hollow plunger, which second plunger can be moved up and  
down and, after the sample fluid has been expelled,  
ensures that washing fluids are extracted from an inlet  
25 which is connected to the valve element by means of a  
non-return valve, and that said washing fluids and poss-  
ibly also the eluant are then passed through the filter  
and the valve element. In the lowest position of the  
hollow plunger the plunger seals off the largest part of  
30 the container relative to the shut-off element, so that  
only a small volume needs to be washed, and a small  
quantity of washing fluid will therefore suffice.

It is also pointed out that, instead of the  
slidable shut-off element shown in Figs. 1 - 6, a rotary  
35 shut-off element can be used. This can be comparable to  
the rotary valve of Fig. 7, but it is also possible to  
form the passage channels as recesses on the periphery of  
the rotary valve. In one position a first recess provides  
the passage to the outlet, while this first recess in a

- 10 -

second position provides the venting, and a second peripheral recess permits the passage of eluant fluid to the eluate reservoir. The needle elements for eluate passage and venting are formed at a position below the  
5 rotary valve in this embodiment.

- 11 -

Claims

1. Device for use in the isolation of a biological material, such as nucleic acid, from a basic material containing said biological material, characterized by a  
5 container (1) for holding a mixture of the basic material, a chaotropic substance and a solid phase which binds the biological material; means (10) for separating the solid phase with the biological material from the fluid bound thereto; and means (3, 8, 12, 13; 28) for  
10 connecting the container (1) to an inlet (6) and outlet (9) for washing fluid for washing the biological material bound to the solid phase, to an inlet (6) for an eluant fluid, and to an eluate reservoir (17) for collection of the eluant fluid with the dissolved biological material.
- 15 2. Device according to Claim 1, for use in a solid phase consisting of particle material, in which the means (10) for separating the solid phase from the fluid are provided with a filter for allowing through the fluid and retaining the particle material.
- 20 3. Device according to Claim 1 or 2, in which the means for connecting the container to the inlet or outlet for fluid and washing fluid and/or to the eluate reservoir are provided with a shut-off element (13, 28).
- 25 4. Device according to Claim 3, in which the shut-off element (13) is provided with a septum (20) and an outlet channel (14) for allowing through fluid from the separating means (1) to the outlet (9), and also a hollow needle element (12) connecting to the separating means  
30 (1), for piercing the septum (20) in order to connect the separating means (1) to the eluate reservoir (17).
5. Device according to Claim 4, in which the needle element (12) is fitted so that it is stationary, and the shut-off element (13) with the septum (18) is fitted so that it can be moved in the direction of the needle  
35 element (12).
6. Device according to one of Claims 2 - 5, in which the filter of the separating means (10) is a filter membrane.

- 12 -

7. Device according to one of Claims 4 - 6, in which the eluate reservoir (17) consists of a container shut off by a second septum (18).

8. Device according to one of Claims 4 - 6, in which  
5 the eluate reservoir (17) is immovably fixed to the shut-off element (13) provided with the septum (20).

9. Device according to one of the preceding claims, provided with an inlet element (23) which can be connected to the inlet (6) and is movable between a top  
10 position for discharging sample fluid and a bottom position for passing through washing fluid and eluant fluid.

10. Device according to Claim 9, in which the container (1) is provided with two sections (25, 26) lying  
15 one above the other and separated by a constriction (24), and the inlet element (23) is movable between a position above the constriction (24) and a position connecting in a close fit to the constriction (24).

11. Device according to Claim 10, in which the inlet  
20 element (23) is in the form of a hollow pin having snap elements (27) at the end for engagement with the constriction (24).

12. Device according to Claim 10 or 11, in which the inlet element (23) is suspended from a flexible cover (2)  
25 of the container (1), which permits the movement of the inlet element (23).

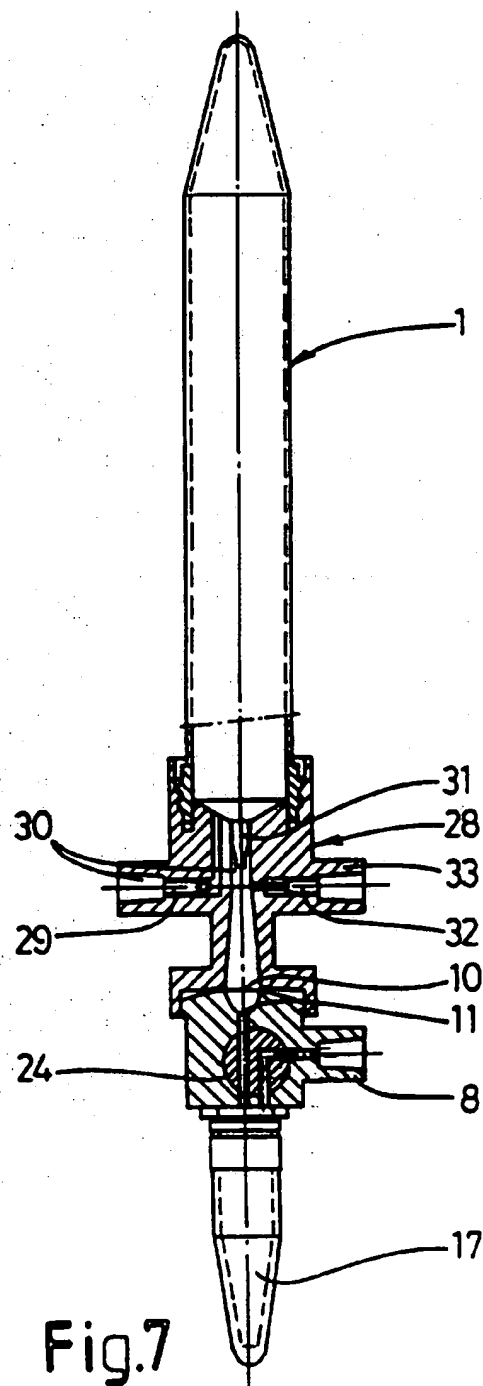
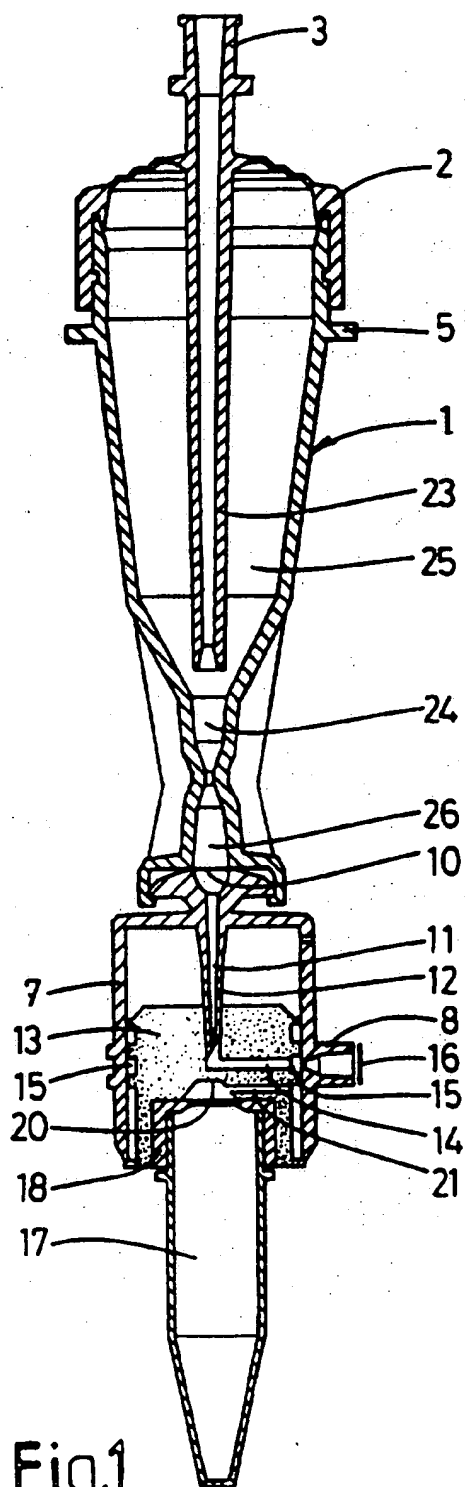
13. Device according to one of Claims 1 - 3, in which the shut-off element (28) is a valve element designed with at least one non-return valve (29) between inlet (9)  
30 and container (1).

14. Device according to Claim 13, in which the valve element (28) is provided with three non-return valves: the first-mentioned non-return valve (29) in the connection (30) to a compressed gas supply, a second non-return  
35 valve (31) between the container (1) and the separating means (10, 11), and a third non-return valve (32) in a connection (33) between the separating means (10, 11) and the inlet for the washing fluid and the eluant fluid.

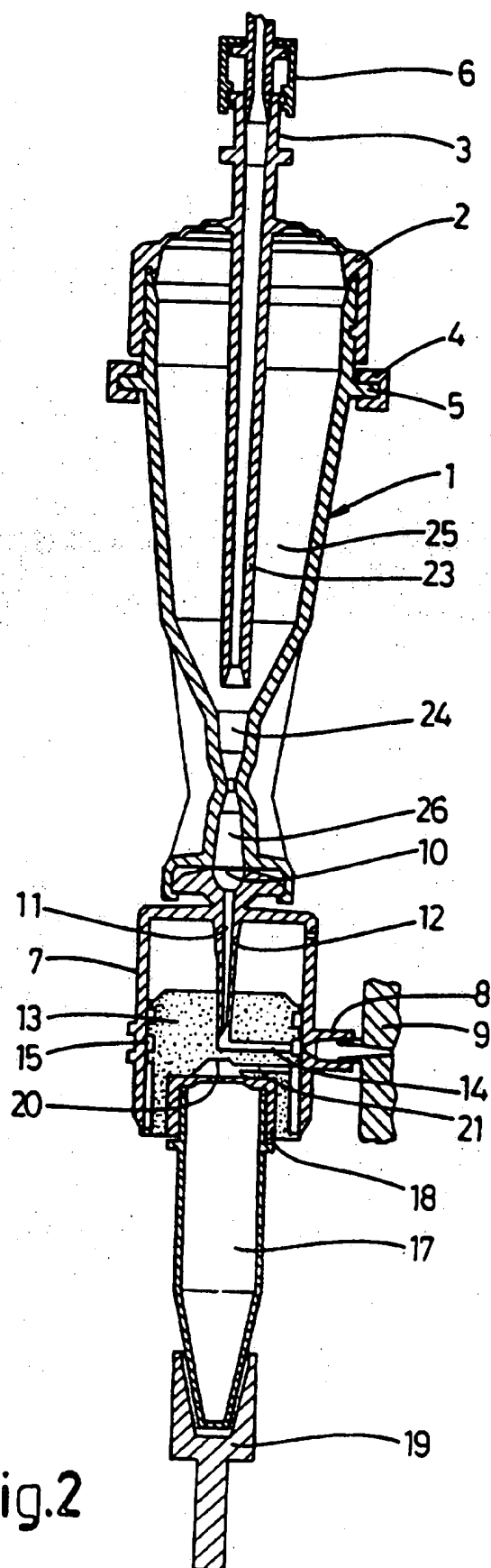
15. Device according to Claim 13 or 14, in which the

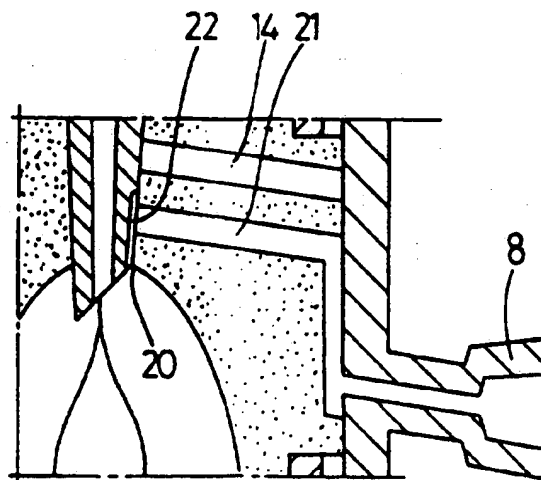
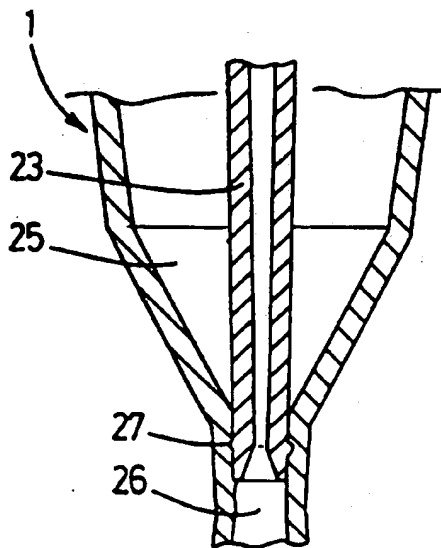
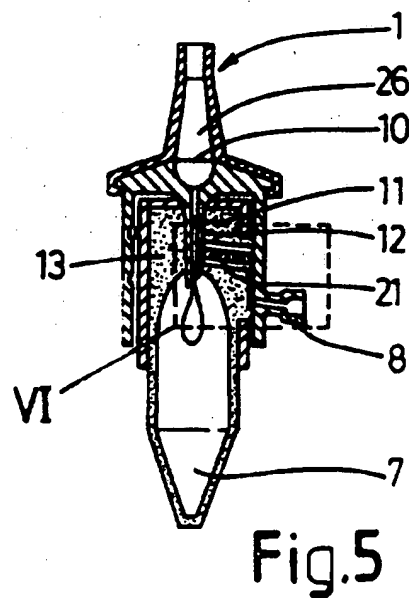
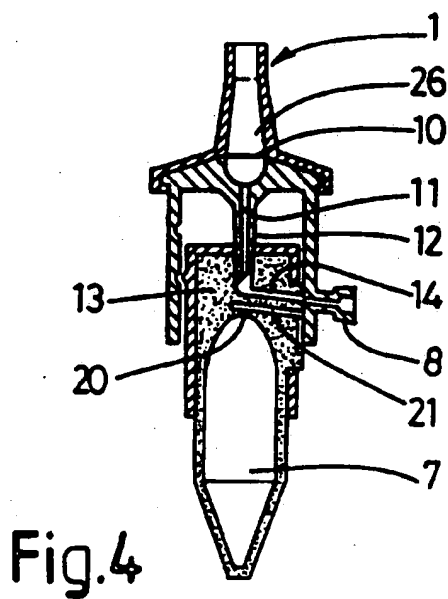
- 13 -

valve element (28) is also provided with a rotary or slide valve (34), for connecting the separating means (10, 11) to the outlet (8, 9) and the eluate reservoir (17) respectively.









# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 95/03385

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07H1/08 G01N33/543 B01D15/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C07H G01N B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y A	US,A,4 812 293 (MCLAURIN) 14 March 1989 see column 3, line 64 - column 8, line 66 ---	1,2 3-8
Y	EP,A,0 389 063 (AKZO) 26 September 1990 cited in the application see page 20; claims 1-15 ---	1,2
A	EP,A,0 141 547 (AMERICAN HOSPITAL SUPPLY) 15 May 1985 see page 5, line 14 - page 10, line 6 ---	1-8
A	EP,A,0 471 570 (GUIRGUIS) 19 February 1992 see page 22-23; claims 1-10 ---	1
A	WO,A,81 00913 (GRAAS) 2 April 1981 see page 9, line 18 - page 10, line 19 -----	1,9,10

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \* "A" document defining the general state of the art which is not considered to be of particular relevance
- \* "E" earlier document but published on or after the international filing date
- \* "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \* "O" document referring to an oral disclosure, use, exhibition or other means
- \* "P" document published prior to the international filing date but later than the priority date claimed

\* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\* "A" document member of the same patent family

Date of the actual completion of the international search

3 January 1996

Date of mailing of the international search report

19 - 01 - 1996

Name and mailing address of the ISA  
European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Wendling, J-P

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT, EP 95/03385

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4812293	14-03-89	NONE	
EP-A-389063	26-09-90	NL-A- 8900725	16-10-90
		AU-B- 641641	30-09-93
		AU-B- 5215390	27-09-90
		CA-A- 2012777	23-09-90
		JP-A- 2289596	29-11-90
		US-A- 5234809	10-08-93
EP-A-141547	15-05-85	US-A- 4786471	22-11-88
		CA-A- 1229300	17-11-87
		JP-A- 60115865	22-06-85
EP-A-471570	19-02-92	US-A- 5137031	11-08-92
		AU-B- 8245291	20-02-92
		CA-A- 2049098	16-02-92
WO-A-8100913	02-04-81	US-A- 4270921	02-06-81
		AU-B- 6242780	09-04-81
		CA-A- 1138219	28-12-82
		EP-A- 0038826	04-11-81
		GB-A, B 2073417	14-10-81

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**